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FILE 'REGISTRY' ENTERED AT 17:36:39 ON 10 APR 2003

E S GRISEUS TRYPSIN/CN

E STREPTOMYCES GRISEUS TRYPSIN/CN

E PRONASE/CN

L1 1 SEA ABB=ON PRONASE/CN  
E TRYPSIN/CN

L2 1 SEA ABB=ON TRYPSIN/CN  
E TRYPSIN (STREPT/CN

L3 1 SEA ABB=ON "TRYPSIN (STREPTOMYCES GRISEUS)"/CN

FILE 'HCAPLUS' ENTERED AT 17:40:12 ON 10 APR 2003

L4 64 SEA ABB=ON L3 OR (S OR ?STREPTOMYCES?) (W)?GRISEUS?(W)?TRYPSIN?

L5 7856 SEA ABB=ON L1 OR ?PRONASE?

L6 22 SEA ABB=ON L4 AND L5

L7 13 SEA ABB=ON L6 AND (?ISOLAT? OR ?PURIF?)

L8 10 SEA ABB=ON L6 AND (?AMIDIN? OR ?GUANADIN? OR ?AMINE?)

L9 19 SEA ABB=ON L7 OR L8 *19 cite in CA Plus*

~~L10 0 SEA ABB=ON L9 AND (?PURIT? OR ?PURIF?) (W) 95~~

FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, JICST-EPLUS, JAPIO' ENTERED AT  
17:46:03 ON 10 APR 2003

L11 11 SEA ABB=ON L9

L12 8 DUP REMOV L11 (3 DUPLICATES REMOVED) *8 cite in other databases*

=&gt; d que stat 19

L1 1 SEA FILE=REGISTRY ABB=ON PRONASE/CN  
 L3 1 SEA FILE=REGISTRY ABB=ON "TRYPSIN (STREPTOMYCES GRISEUS)"/CN  
 L4 64 SEA FILE=HCAPLUS ABB=ON L3 OR (S OR ?STREPTOMYCES?) (W)?GRISEUS  
 ?(W)?TRYPSIN?  
 L5 7856 SEA FILE=HCAPLUS ABB=ON L1 OR ?PRONASE?  
 L6 22 SEA FILE=HCAPLUS ABB=ON L4 AND L5  
 L7 13 SEA FILE=HCAPLUS ABB=ON L6 AND (?ISOLAT? OR ?PURIF?)  
 L8 10 SEA FILE=HCAPLUS ABB=ON L6 AND (?AMIDIN? OR ?GUANADIN? OR  
 ?AMINE?)  
 L9 19 SEA FILE=HCAPLUS ABB=ON L7 OR L8

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L9 ANSWER 1 OF 19 HCAPLUS COPYRIGHT 2003 ACS  
 ACCESSION NUMBER: 1998:643707 HCAPLUS  
 DOCUMENT NUMBER: 130:34822  
 TITLE: **Purification** and characterization of  
**Streptomyces griseus trypsin**  
 overexpressed in *Streptomyces lividans*  
 AUTHOR(S): Koo, Bon-Joon; Bae, Kwang Hee; Byun, Si-Myong; Hong,  
 Soon-Kwang  
 CORPORATE SOURCE: Department of Biological Science, Myong Ji University,  
 Kyonggi-Do, S. Korea  
 SOURCE: Journal of Microbiology and Biotechnology (1998),  
 8(4), 333-340  
 CODEN: JOMBES; ISSN: 1017-7825  
 PUBLISHER: Korean Society for Applied Microbiology  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB **Streptomyces griseus trypsin** (SGT) is an  
 extracellular proteinase produced by *S. griseus*. The sprT gene, which  
 encodes premature SGT protein, was cloned into the plasmid pWHM3, a  
*Streptomyces-E. coli* shuttle vector. When the recombinant plasmid was  
 introduced into *Streptomyces lividans* TK24, two proteins with mol. wts. of  
 28 kDa and 42 kDa were detected. The 28-kDa protein was a SGT protein  
 while the larger 42-kDa protein is thought to have been a premature form  
 of the SGT protein. The SGT protein was **purified** to homogeneity  
 via ammonium sulfate fractionation and many column chromatogs., including  
 CM-sepharose chromatog., Mono-S chromatog., and Superose-12 chromatog.,  
 from the culture broth of *S. lividans* TK24 harboring the sprT gene. The  
 N-terminal amino acid sequence, isoelec. points, and stabilities at  
 various conditions of the SGT proteins **purified** from the  
**Pronase** and transformant were almost identical. The amt. of the  
 expressed SGT in *S. lividans* TK 24 was detd. to be 5 times more than that  
 of *S. griseus* based on the enzymic activity against artificial substrate.  
 REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS  
 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 2 OF 19 HCAPLUS COPYRIGHT 2003 ACS  
 ACCESSION NUMBER: 1988:18250 HCAPLUS  
 DOCUMENT NUMBER: 108:18250  
 TITLE: High-performance affinity adsorbents for  
 trypsin-family enzymes prepared with TSKgel G3000PW  
 AUTHOR(S): Kanamori, Akiko; Seno, Nobuko; Matsumoto, Isamu  
 CORPORATE SOURCE: Fac. Sci., Ochanomizu Univ., Tokyo, 112, Japan  
 SOURCE: Chemical & Pharmaceutical Bulletin (1987), 35(9),

3777-83

CODEN: CPBTAL; ISSN: 0009-2363

DOCUMENT TYPE:

Journal

LANGUAGE:

English

AB For affinity HPLC (HPLAC) of trypsin-family enzymes, 2 kinds of adsorbents (I and II) were prepd. by convenient procedures using m-**aminobenzamidine** (ABA) and TSKgel G3000PW, a carrier for HPLC. For adsorbent I, ABA was immobilized on succinyl-TSKgel G3000PW, which was prepd. by successive epoxy activation, amination, and succinylation of TSKgel G3000PW with the aid of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (.apprx.16 .mu.mol ABA/g wet gel; .apprx.80% yield). For adsorbent II, ABA was immobilized on a formyl carrier, which was prepd. by successive epoxy activation, hydrolysis, and periodate-oxidn. of TSKgel G3000PW through reductive amination with NaCNBH3 (19 .mu.mol ABA/g wet gel; 98% yield). In the case of HPLAC on adsorbent I, bovine and **Streptomyces griseus** trypsins were eluted at 12 and 19 min, resp., whereas the enzymes were retained on adsorbent II and were recovered efficiently with eluting buffer contg. 6-aminohexanoic acid. Thus, adsorbent I is useful for the rapid anal. of the enzymes and adsorbent II for the prepn. of the enzymes.

L9 ANSWER 3 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1986:586397 HCAPLUS

DOCUMENT NUMBER: 105:186397

TITLE: Preparation of high-capacity affinity adsorbents using formyl carriers and their use for low- and high-performance liquid affinity chromatography of trypsin-family proteases

AUTHOR(S): Kanamori, Akiko; Seno, Nobuko; Matsumoto, Isamu

CORPORATE SOURCE: Fac. Sci., Ochanomizu Univ., Tokyo, 112, Japan

SOURCE: Journal of Chromatography (1986), 363(2), 231-42

CODEN: JOCRAM; ISSN: 0021-9673

DOCUMENT TYPE:

Journal

LANGUAGE:

English

AB To prep. stable and high-capacity affinity adsorbents for trypsin-family proteases, high concns. of m-**aminobenzamidine** (ABA) were immobilized by reductive amination with NaCNBH4 on 2 types of formyl carriers, I and II, having different spacer lengths. Formyl carriers I were prepd. by periodate oxidn. of glucose carriers obtained by coupling glucose to epoxy-activated carriers. Formyl carriers II were prepd. by periodate oxidn. of glyceryl carriers obtained by hydrolysis of epoxy-activated carriers. High concns. of ABA (15-21 .mu.mol/g wet gel) were efficiently immobilized on both types of carriers, and formyl groups remaining on the adsorbents were converted into hydroxymethyl groups by redn. with NaBH4. Two types of adsorbents prepd. with Sepharose gel were successfully used for affinity chromatog. of bovine trypsin, **Streptomyces griseus** trypsin, and serine proteases in urine, and exhibited high adsorption capacities, e.g., .apprx.30 mg bovine trypsin/mL gel. Adsorbents prepd. with Toyopearl gel (Fractogel TSK) were also used successfully for HPLC of bovine trypsin, though they exhibited lower adsorption capacities than those prepd. with Sepharose gels.

L9 ANSWER 4 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1986:454423 HCAPLUS

DOCUMENT NUMBER: 105:54423

TITLE: Intradiscal enzyme therapy: dissolution of proteoglycans or collagen?

AUTHOR(S): Tavakol, K.; Waisman, D. M.; LeBlanc, F. E.; Schachar, N. S.

CORPORATE SOURCE: Fac. Med., Univ. Calgary, Calgary, AB, T2N 4N1, Can.  
SOURCE: Current Therapeutic Research (1986), 39(6), 924-32  
CODEN: CTCEA9; ISSN: 0011-393X

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB To study the mechanism of dissoln. of the insol. portion of herniated nucleus pulposus(NP), the ability and potency of 15 proteases to solubilize the major components of human NP collagen, and proteoglycans (PG) was detd. Following NP proteolysis in vitro and using a math. model, the max. levels of hydroxyproline and hexosamine release were detd. as markers of collagen and PG dissoln., resp., and the corresponding percentages of NP wt. loss were detd. as indicators of NP dissoln. Proteinase-K [39450-01-6], protease [9001-92-7] from *Bacillus subtilis* and *Streptomyces griseus*, trypsin [9002-07-7], pronase [9036-06-0], papain [9001-73-4], bacterial collagenase A (COLL) [9001-12-1], and chymopapain (CHYM) [9001-09-6] solubilized both PG and collagen, and resulted in substantial amts. of NP wt. loss, *Staphylococcus aureus* and *Submaxilaris* proteases, .alpha.-chymotrypsin [9004-07-3], carboxypeptidase A [11075-17-5] and carboxypeptidase B [9025-24-5], neuraminidase [9001-67-6], and leucine aminopeptidase [9001-61-0] dissolved only PG in small amts., showing low levels of NP wt. loss. The ability of the enzymes to dissolve human NP correlates more with solubilization of PG than collagen, the enzymes that degrade both collagen and PG have greater potency to dissolve NP than those that degrade only PG, and most of the enzymes tested were more potent than CHYM and almost as potent as COLL.

L9 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1985:556271 HCAPLUS

DOCUMENT NUMBER: 103:156271

TITLE: High-performance affinity chromatography of trypsins on Asahipak GS-gel coupled with p-aminobenzamidine

AUTHOR(S): Ito, Naofumi; Noguchi, Kohji; Shimura, Kiyohito; Kasai, Kenichi

CORPORATE SOURCE: Technol. Dev. Dep., Asahi Chem. Ind. Co. Ltd., Kawasaki, 210, Japan

SOURCE: Journal of Chromatography (1985), 333(1), 107-14  
CODEN: JOCRAM; ISSN: 0021-9673

DOCUMENT TYPE: Journal

LANGUAGE: English

AB An adsorbent for affinity HPLC of trypsins was prepd., based on a micro-particulate polyvinyl alc. gel, Asahipak GS-gel. After the OH groups had been activated with 1,1'-carbonyldiimidazole, 6-aminohexanoic acid was coupled as a spacer; then p-aminobenzamide, a specific ligand for trypsin-family enzymes, was immobilized on the spacer. Fluorometric detection of eluted protein and on-line assay of enzyme activity with a fluorogenic substrate, peptidylmethylcoumaryl amide, made it possible to attain very high sensitivity. Microgram amts. of bovine trypsin and *Streptomyces griseus* trypsin could easily be analyzed in a short time (<1 h).

L9 ANSWER 6 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1985:108564 HCAPLUS

DOCUMENT NUMBER: 102:108564

TITLE: Introduction of .omega.-carboxyl spacers onto cross-linked agarose gel beads by O-alkylation for the preparation of affinity adsorbents

AUTHOR(S): Shimura, Kiyohito; Kasai, Kenichi

CORPORATE SOURCE: Fac. Pharm. Sci., Teikyo Univ., Kanagawa, 199-01,

SOURCE: Japan  
Journal of Chromatography (1984), 315, 161-6  
CODEN: JOCRAM; ISSN: 0021-9673  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Crosslinked agarose gel beads (Sephacrose CL-4B) were O-alkylated with chloroacetylglutylglycine or N-chloroacetyl-6-aminohexanoic acid in DMSO using methylsulfinyl carbanion as a catalyst. The reaction was completed within 30 min, and the degree of substitution reached 100  $\mu\text{mol/mL}$  settled vol. of the gel with  $\approx 50\%$  yield. The terminal  $\text{CO}_2\text{H}$  group of the spacers can be coupled with an affinity ligand contg. an  $\text{NH}_2$  function using water-sol. carbodiimide. The ligand-matrix linkage is stable and free from undesired elec. charge. **Streptomyces griseus Trypsin** was purified to homogeneity from **Pronase** using a column of the Sepharose deriv. coupled with p-aminobenzamidine.

L9 ANSWER 7 OF 19 HCAPLUS COPYRIGHT 2003 ACS  
ACCESSION NUMBER: 1985:41904 HCAPLUS  
DOCUMENT NUMBER: 102:41904  
TITLE: Affinophoresis of trypsins with an anionic affinophore  
AUTHOR(S): Shimura, Kiyohito; Kasai, Kenichi  
CORPORATE SOURCE: Fac. Pharm. Sci., Teikyo Univ., Kanagawa, 199-01, Japan  
SOURCE: Biochimica et Biophysica Acta (1984), 802(1), 135-40  
CODEN: BBACAQ; ISSN: 0006-3002  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB An anionic affinophore for trypsin affinophoresis (Shimura, K.; Kasai, K., 1982) was synthesized. p-Aminobenzamidine, a competitive inhibitor of trypsin, was coupled to one-fifth of the carboxyl groups of polyacrylyl-.beta.-alanyl-.beta.-alanine by the use of water-sol. carbodiimide and the residual carboxyls were converted to sulfonate groups by coupling with aminomethanesulfonic acid. Affinophoresis was carried out in 1% agarose gel plates, and the protein bands were detected with Coomassie brilliant blue R250. Enhanced migrations of bovine and **Streptomyces griseus trypsins** towards the anode were obsd. with the anionic affinophore. The migrations of inactive forms prepd. by active site modifications were scarcely affected. However, the affinophore was not effective for *S. erythraeus* trypsin, an anionic trypsin, probably because of ionic repulsion between the anionic mols. **S. griseus Trypsin** was sepd. from **Pronase** by affinophoresis.

L9 ANSWER 8 OF 19 HCAPLUS COPYRIGHT 2003 ACS  
ACCESSION NUMBER: 1983:211944 HCAPLUS  
DOCUMENT NUMBER: 98:211944  
TITLE: Light sensitization of a microbial protease  
AUTHOR(S): Kuan, Kenneth N.; Lee, Y. Y.; Melius, Paul  
CORPORATE SOURCE: Dep. Chem. Eng., Auburn Univ., Auburn, AL, 36849, USA  
SOURCE: Journal of Applied Biochemistry (1982), 4(4), 384-90  
CODEN: JABIDV; ISSN: 0161-7354  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB A trypsin-like enzyme from a com. prepn. of *Streptomyces griseus* **Pronase** was purified by successive affinity chromatog. on carbobenzoxy-L-phenylalanyl-triethylenetetraminyl-Sepharose and CM-cellulose 52 ion exchanger. This microbial enzyme possessed a unique characteristic in that it could be artificially light-sensitized by acylation with cis-cinnamoylimidazole.

L9 ANSWER 9 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1983:49288 HCAPLUS

DOCUMENT NUMBER: 98:49288

TITLE: Affinophoresis of trypsins

AUTHOR(S): Shimura, Kiyohito; Kasai, Kenichi

CORPORATE SOURCE: Fac. Pharm. Sci., Teikyo Univ., Sagamiko, 199-01, Japan

SOURCE: Journal of Biochemistry (Tokyo, Japan) (1982), 92(5), 1615-22

CODEN: JOBIAO; ISSN: 0021-924X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A new sepn. technique for proteins, named affinophoresis, which is based on its specific affinity and utilizes electrophoresis, was devised. This technique requires a carrier macromol., affinophore, which contains both an affinity ligand for a certain protein and many charges, either pos. or neg., in order to migrate rapidly in an elec. field. When a mixt. of proteins is electrophoresed in the presence of the affinophore, the protein having an affinity with the ligand will form a complex with the affinophore. This results in a change in the apparent electrophoretic mobility. If the protein mobility is sufficiently accelerated, it can be sepd. from other materials. A cationic affinophore for trypsin was prepd. Sol. dextran (mol. wt. .apprx.10,000) was coupled with a DEAE group and m-aminobenzamidine, a competitive inhibitor of trypsin.

Electrophoresis of trypsins from several sources on agarose gel plates in the presence of the affinophore showed that affinophoresis actually occurred. The electrophoretic mobilities of the trypsin mols. increased towards the cathode, the same direction as the affinophore movement. The presence of leupeptin and treatment of the trypsins with TLCK suppressed the effect of the affinophore. **Streptomyces griseus** Trypsin, contained in **Pronase**, was easily sepd. and detected. This procedure is distinct from affinity chromatog. and so-called affinity electrophoresis in that the support of the affinity ligand moves, and has advantages esp. for anal. purposes: e.g., the detection of specific mols., regardless of their isoelec. points.

L9 ANSWER 10 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1981:152455 HCAPLUS

DOCUMENT NUMBER: 94:152455

TITLE: Rapid purification of **Streptomyces griseus** trypsin by immobilized rice bran trypsin inhibitor

AUTHOR(S): Tashiro, Misao; Sugihara, Nobuo; Maki, Zensuke; Kanamori, Masao

CORPORATE SOURCE: Fac. Living Sci., Kyoto Prefect. Univ., Kyoto, 606, Japan

SOURCE: Agricultural and Biological Chemistry (1981), 45(2), 519-21

CODEN: ABCHA6; ISSN: 0002-1369

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The trypsin activity of *S. griseus* **Pronase-P** was purified to homogeneity on an affinity column of rice bran trypsin inhibitor immobilized on CNBr-activated Sepharose 4B. The enzyme was eluted at acidic pH and showed only 1 component on SDS-gel electrophoresis. Purified trypsin had a relatively high sp. activity for trypsin substrates and no exopeptidase activity. The preparative method was rapid and highly efficient.

L9 ANSWER 11 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1976:401636 HCAPLUS

DOCUMENT NUMBER: 85:1636

TITLE: Affinity chromatography of trypsin and related enzymes. III. Purification of **Streptomyces griseus trypsin** using an affinity adsorbent containing a tryptic digest of **protamine** as a ligand

AUTHOR(S): Yokosawa, Hideyoshi; Hanba, Takayoshi; Ishii, Shinichi  
CORPORATE SOURCE: Fac. Pharm. Sci., Hokkaido Univ., Sapporo, Japan  
SOURCE: Journal of Biochemistry (Tokyo, Japan) (1976), 79(4), 757-63

CODEN: JOBIAO; ISSN: 0021-924X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A new, simple method was developed for the purifn. of **S. griseus trypsin** (EC 3.4.21.4) from **Pronase**. Only a single operation of affinity chromatog. on an agarose deriv., which was easily prepd. by coupling a tryptic digest of salmine to CNBr-activated Sepharose 4B, was required. A high degree of homogeneity was demonstrated for the **purified** enzyme by disc electrophoresis, Na dodecyl sulfate-polyacrylamide gel electrophoresis, and gel filtration, as well as by active site titrn. The behavior of a carboxypeptidase B (EC 3.4.12.3)-like enzyme present in **Pronase** is also discussed.

L9 ANSWER 12 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1975:403308 HCAPLUS

DOCUMENT NUMBER: 83:3308

TITLE: Enzymic and physicochemical properties of **Streptomyces griseus trypsin**

AUTHOR(S): Olafson, Robert W.; Smillie, Lawrence B.  
CORPORATE SOURCE: Dep. Biochem., Univ. Alberta, Edmonton, AB, Can.  
SOURCE: Biochemistry (1975), 14(6), 1161-7

CODEN: BICHAW; ISSN: 0006-2960

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Streptomyces griseus trypsin** was isolated from **Pronase** by ion-exchange chromatog. on CM-Sephadex and SE-Sephadex. The **isolated** enzyme was homonogeneous by the criteria except for a low degree of contamination by an enzyme with nontryptic activity. The latter could be partially resolved by chromatog. on Bio-Rex 70. The molar absorbancy at 280 nm was 3.96 .times. 10<sup>-6</sup>M-1/cm and the Elcm1% was 17.3. The mol. wt. was 22,800 .+- 800. The enzyme was stable at 0.degree. from pH 2 to 10. At 30.degree. the enzyme was maximally stable at pH 3-4 and significantly stabilized in the neutral and alk. range by 15mM Ca<sup>2+</sup>. Some evidence was obtained for a reversible denaturation of the enzyme at pH 12.0 and 2.0. The Km for N-.alpha.-benzoyl-L-arginine Et ester at pH 8.0 in 20mM CaCl<sub>2</sub>-0.1M KCl-10mM Tris-HCl buffer at 30.degree. was 7.7 .+- 1.9 .times. 10<sup>6</sup>M and the esterase activity was dependent on an ionizing group with pKa = 5.85. In 2H<sub>2</sub>O this pKa was increased to 6.35 and the rate of hydrolysis decreased 3-fold. The rate of hydrolysis was independent of pH between 8 and 10. The inhibition of the enzyme with L-1-chloro-3-tosylamido-4-phenyl-2-butanone was assocd. with the alkylation of its single histidine residue. This residue is present in a homologous amino acid sequence as the active-site histidine in trypsin and chymotrypsin. ORD and CD measurements over the pH range 5.3-10.5 indicated no significant conformational change until the pH was increased >10.1. The observation that, under the conditions tested, acetylation and carbamylation of the

N-terminal valine were incomplete is consistent with the view that this group is buried as an ion pair and only becomes available for deprotonation and reaction upon denaturation of the enzyme at pH values >10.0.

L9 ANSWER 13 OF 19 HCAPLUS COPYRIGHT 2003 ACS  
ACCESSION NUMBER: 1974:487173 HCAPLUS  
DOCUMENT NUMBER: 81:87173  
TITLE: Amino acid sequence of **Streptomyces griseus trypsin**. II. Tryptic peptides  
AUTHOR(S): Jurasek, L.; Smillie, L. B.  
CORPORATE SOURCE: Dep. Biochem., Univ. Alberta, Edmonton, AB, Can.  
SOURCE: Canadian Journal of Biochemistry (1974), 52(5), 382-92  
CODEN: CJBIAE; ISSN: 0008-4018  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB **S. griseus trypsin** (S.G.T.) isolated from **Pronase** was reduced, aminoethylated, and digested with trypsin. The sol. peptides were recovered and the insol. residue redigested with chymotrypsin. Following recovery of the sol. fraction, the insol. portion was in turn digested with .alpha.-lytic protease of Myxobacter 495. The 3 groups of sol. peptides were sep. subjected to ion-exchange chromatog. on the Technicon peptide analyzer and to final **purifn.** by high-voltage paper electrophoresis. Sequence anal. by the dansyl-Edman procedure provided unique sequences from the sol. tryptic peptides accounting for 65% of the S.G.T. mol. Peptides obtained from the redigests of the insol. residues accounted for an addnl. 20%. Tryptic digestion of dansylated S.G.T. yielded a unique .alpha.-NH2 dansylated peptide whose compn. showed it to be the same as the NH2-terminal sequence previously postulated for this enzyme. The tryptic peptides provided overlaps for many of the previously sequenced peptic peptides. Three continuous sequences of 49, 36, and 28 residues were elucidated. Evidence was also obtained that the NH2-terminal valine residue was, to a limited extent, aminoethylated during reaction of the reduced protein with ethylenimine.

L9 ANSWER 14 OF 19 HCAPLUS COPYRIGHT 2003 ACS  
ACCESSION NUMBER: 1973:533883 HCAPLUS  
DOCUMENT NUMBER: 79:133883  
TITLE: Proteolytic enzymes of the K1 strain of **Streptomyces griseus** obtained from a commercial preparation (**Pronase**). **Purification** and characterization of the aminopeptidases  
AUTHOR(S): Vosbeck, Klaus D.; Chow, Kai-Fu; Awad, William M., Jr.  
CORPORATE SOURCE: Sch. Med., Univ. Miami, Miami, FL, USA  
SOURCE: Journal of Biological Chemistry (1973), 248(17), 6029-34  
CODEN: JBCHA3; ISSN: 0021-9258  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB An affinity matrix, 1,6-diaminohexane-agarose, was synthesized and demonstrated to bind **S. griseus trypsin**. This agarose deriv. also bound the 2 components with aminopeptidase activity present in **Pronase**. The trypsin was completely sepd. from the aminopeptidases by initial passage through a CM-cellulose column. Thereafter, the trypsin was selectively retarded by the **hexanediamine**-agarose; all assocd. components were eluted with 1M NaCl at pH 8, whereas the trypsin could be eluted only after application of an acidic (pH 3) buffer. This enzyme appeared to be homogeneous by



other studies. Bovine trypsin was not retarded by this chromatog. procedure. The aminopeptidases were **purified** by passage through the same column. Their complete selective retardation was achieved only after prior treatment with Na **ethylenediaminetetraacetate**. Ca<sup>2+</sup> at low concns. eluted the 2 enzymes sep. Ca<sup>2+</sup> was required for the activity of each enzyme and also stabilized each enzyme against heat denaturation. Sr<sup>2+</sup> restored about 2/3 of the activities to the metal-free proteins as compared to the activities noted with Ca<sup>2+</sup>; other divalent cations provided much less activity. The max. activity of each aminopeptidase was between pH values of 7.5 and 10; each enzyme was stable between pH values of 6 and 11. These enzymes were tentatively designated aminopeptidase 1 and aminopeptidase 2 after the order of their elution from **hexanedi-amine**-agarose; gel filtration revealed their approx. mol. wts. to be 23,000 and 25,000, resp. A single band was seen for each protein after acrylamide gel electrophoresis.

L9 ANSWER 15 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1973:475429 HCAPLUS

DOCUMENT NUMBER: 79:75429

TITLE: Amino acid sequence of **Streptomyces griseus trypsin**. I. Peptic peptides

AUTHOR(S): Jurasek, L.; Smillie, L. B.

CORPORATE SOURCE: Dep. Biochem., Univ. Alberta, Edmonton, AB, Can.

SOURCE: Canadian Journal of Biochemistry (1973), 51(7), 1077-88

CODEN: CJBIAE; ISSN: 0008-4018

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Trypsin of *S. griseus* **isolated** from **Pronase** was digested with pepsin. The peptic peptides were **isolated** by high-voltage electrophoresis on paper and ion-exchange chromatog. Anal. of the **purified** peptides provided 28 unique amino acid sequences accounting for .apprx.95% of the trypsin mol. A portion of the residues not accounted for were ascribed to free leucine, phenylalanine, tryrosine, and tryptophan present in the peptic digest. The N-terminal sequence of trypsin was Val-Val-Gly-Gly-Thr-Arg-Ala-Ala-Gln-Gly-Glu-Phe and was highly homologous with N-terminal sequences of other Asp-Ser-Gly serine proteases.

L9 ANSWER 16 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1973:54830 HCAPLUS

DOCUMENT NUMBER: 78:54830

TITLE: Antibody and inhibitor interactions with **pronase trypsin**

AUTHOR(S): Trop, M.; Pinsky, A.; Avtalion, R.

CORPORATE SOURCE: Dep. Life Sci., Bar-Ilan Univ., Ramat-Gan, Israel

SOURCE: Immunology (1972), 22(4), 531-5

CODEN: IMMUAM; ISSN: 0019-2805

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Antibody to **Pronase** trypsin reacts with its antigenic sites to the same extent whether the enzyme is a sol. complex with its inhibitor or is free. The inhibitor reacts poorly with the antibody-bound enzyme. Excess of antibody displaces the insol. inhibitor from its complex with the enzyme. This displacement effect is greater when the inhibitor is bound to agarose by means of CNBr treatment rather than by means of **ethylenediamine** glutaraldehyde. It is assumed, therefore, that there is competition between the antibody and the inhibitor for the plane tangent to the active site of the enzyme, but not for the active site

itself or for its immediate vicinity.

L9 ANSWER 17 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1972:22505 HCAPLUS

DOCUMENT NUMBER: 76:22505

TITLE: Improved fractionation system for **pronase** on CM-Sephadex

AUTHOR(S): Jurasek, L.; Johnson, P.; Olafson, R. W.; Smillie, L. B.

CORPORATE SOURCE: Dep. Biochem., Univ. Alberta, Edmonton, AB, Can.

SOURCE: Canadian Journal of Biochemistry (1971), 49(11), 1195-201

CODEN: CJBIAE; ISSN: 0008-4018

DOCUMENT TYPE: Journal

LANGUAGE: English

AB An efficient, single-column preparative-scale fractionation of **pronase** on CM-Sephadex using a linear gradient of pyridine-acetate, pH 5.0, has been developed. Under these conditions excellent resolution and minimal autolysis of the component proteolytic enzymes occurs. Only 3 major endopeptidases with caseinolytic activity are found. **Streptomyces griseus trypsin** (S.G.T.) is recovered in adequate purity for amino acid sequence studies. S. griseus protease A and protease B have been shown to correspond to PNPase I and II previously described by Waehlby. Two aminopeptidases and a carboxypeptidase have also been demonstrated. **Pronase** appears to be a less complex mixt. of proteolytic enzymes than had previously been appreciated.

L9 ANSWER 18 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1970:338 HCAPLUS

DOCUMENT NUMBER: 72:338

TITLE: Remarkable homology about the disulfide bridges of a trypsin-like enzyme from *Streptomyces griseus*

AUTHOR(S): Jurasek, Lubomir; Fackre, D.; Smillie, Lawrence B.

CORPORATE SOURCE: Univ. Alberta, Edmonton, AB, Can.

SOURCE: Biochemical and Biophysical Research Communications (1969), 37(1), 99-105

CODEN: BBRCA9; ISSN: 0006-291X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A trypsin-like enzyme cleaving only the arginine and lysine bonds of the oxidized chain of insulin has been **isolated** from com.

**Pronase**. Amino acid anal. showed only 1 histidine and 6 half-cystine residues and detn. of the sequences about the 3 disulfide bridges showed a remarkable homology with 3 corresponding bridges in bovine pancreatic trypsin.

L9 ANSWER 19 OF 19 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1969:368 HCAPLUS

DOCUMENT NUMBER: 70:368

TITLE: The trypsinlike enzyme from *Streptomyces griseus* (**Pronase**)

AUTHOR(S): Trop, Moshe; Birk, Yehudith

CORPORATE SOURCE: Bar-Ilan Univ., Ramat-Gan, Israel

SOURCE: Biochemical Journal (1968), 109(3), 475-6

CODEN: BIJOAK; ISSN: 0264-6021

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Pronase** was fractionated on a CM-cellulose column with pH and concn. gradient of NH<sub>4</sub>OAc (0.01M, pH 4.6, to 0.2M, pH 6.9). Five

fractions were **isolated** by their activity in hydrolyzing N.alpha.-benzoyl-L-arginine Et ester (I), N-acetyltyrosine Et ester, and casein and the extent of inhibition of activity by trypsin inhibitors. Four fractions had proteolytic activity, but only fraction C hydrolyzed I and was inhibited by trypsin inhibitors. After rechromatog. as before, fraction C was stable in the cold after freeze-drying. It migrated as a single band during electrophoresis at pH 4.5 in polyacrylamide gel, and eluted as a single peak during chromatog. on Sephadex G-25, G-75, and G-100, and on DEAE-cellulose. The complex which the enzyme formed with trypsin inhibitor AA was dissocd., with recovery of both enzyme and inhibitor activities, after paper electrophoresis for 8 hrs. in pyridine-acetate buffer (0.2M, pH 4.9).

=> d que stat l12

L1 1 SEA FILE=REGISTRY ABB=ON PRONASE/CN  
 L3 1 SEA FILE=REGISTRY ABB=ON "TRYPSIN (STREPTOMYCES GRISEUS)"/CN  
 L4 64 SEA FILE=HCAPLUS ABB=ON L3 OR (S OR ?STREPTOMYCES?) (W)?GRISEUS  
 ?(W)?TRYPSIN?  
 L5 7856 SEA FILE=HCAPLUS ABB=ON L1 OR ?PRONASE?  
 L6 22 SEA FILE=HCAPLUS ABB=ON L4 AND L5  
 L7 13 SEA FILE=HCAPLUS ABB=ON L6 AND (?ISOLAT? OR ?PURIF?)  
 L8 10 SEA FILE=HCAPLUS ABB=ON L6 AND (?AMIDIN? OR ?GUANADIN? OR  
 ?AMINE?)  
 L9 19 SEA FILE=HCAPLUS ABB=ON L7 OR L8  
 L11 11 SEA L9  
 L12 8 DUP REMOV L11 (3 DUPLICATES REMOVED)

=> d ibib abs l12 1-8

L12 ANSWER 1 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1  
 ACCESSION NUMBER: 1998:478891 BIOSIS  
 DOCUMENT NUMBER: PREV199800478891  
 TITLE:

**Purification and characterization of  
 Streptomyces griseus trypsin**  
 overexpressed in Streptomyces lividans.

AUTHOR(S): Koo, Bon-Joon; Bae, Kwang Hee; Byun, Si-Myong; Hong,  
 Soon-Kwang (1)  
 CORPORATE SOURCE: (1) Dep. Biol. Sci., Myong Ju Univ., San 38-2, Nam-Dong,  
 Yongin-City, Kyonggi-Do South Korea  
 SOURCE: Journal of Microbiology and Biotechnology, (Aug., 1998)  
 Vol. 8, No. 4, pp. 333-340.  
 ISSN: 1017-7825.

DOCUMENT TYPE: Article  
 LANGUAGE: English

AB **Streptomyces griseus trypsin** (SGT) is an  
 extracellular proteinase produced by *S. griseus*. The sprT gene, which  
 encodes premature SGT protein, was cloned into the plasmid pWHM3, a  
*Streptomyces*-*E. coli* shuttle vector. When the recombinant plasmid was  
 introduced into *Streptomyces lividans* TK24, two proteins with molecular  
 weights of 28 kDa and 42 kDa were detected. The 28-kDa protein was a SGT  
 protein while the larger 42-kDa protein is thought to have been a  
 premature form of the SGT protein. The SGT protein was **purified**  
 to homogeneity via ammonium sulfate fractionation and many column  
 chromatographies, including CM-sepharose chromatography, Mono-S  
 chromatography, and Superose-12 chromatography, from the culture broth of  
*S. lividans* TK24 harboring the sprT gene. The N-terminal amino acid  
 sequence, isoelectric points, and stabilities at various conditions of the  
 SGT proteins **purified** from the **Pronase** and  
 transformant were almost identical. The amount of the expressed SGT in *S.*  
*lividans* TK 24 was determined to be 5 times more than that of *S. griseus*  
 based on the enzymatic activity against artificial substrate.

L12 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
 ACCESSION NUMBER: 1985:302979 BIOSIS  
 DOCUMENT NUMBER: BA79:82975  
 TITLE: INTRODUCTION OF OMEGA CARBOXYL SPACERS ONTO CROSS-LINKED  
 AGAROSE GEL BEADS BY O ALKYLATION FOR THE PREPARATION OF  
 AFFINITY ADSORBENTS.  
 AUTHOR(S): SHIMURA K; KASAI K-I  
 CORPORATE SOURCE: DEP. BIOLOGICAL CHEMISTRY, FAC. PHARMACEUTICAL SCI., TEIKYO  
 UNIV., SAGAMIKO, KANAGAWA 199-01, JAPAN.  
 SOURCE: J CHROMATOGR, (1984 (RECD 1985)) 315 (0), 161-166.

CODEN: JOCRAM. ISSN: 0021-9673.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB Cross-linked agarose gel beads (Sephacrose CL-4B) were O-alkylated with chloroacetylglutylglycine or N-chloroacetyl-6-aminohexanoic acid in dimethyl sulfoxide by using methylsulfinyl carbanion as a catalyst. The reaction was completed within 30 min and the degree of substitution reached 100  $\mu\text{mol}$  per ml settled volume of the gel with about 50% yield. The terminal carboxyl group of the spacers can be coupled with an affinity ligand containing an amino function by the use of water-soluble carbodiimide. The ligand-matrix linkage was stable and free from undesired electric charge. **Streptomyces griseus trypsin** was purified to homogeneity from **pronase** by using a column of the Sepharose derivative coupled with p-aminobenzamidine

L12 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 1985:277930 BIOSIS  
DOCUMENT NUMBER: BA79:57926  
TITLE: AFFINOPHORESIS OF TRYPSINS WITH AN ANIONIC AFFINOPHORE.  
AUTHOR(S): SHIMURA K; KASAI K-I  
CORPORATE SOURCE: DEP. BIOL. CHEM., FACULTY PHARMACEUTICAL SCI., TEIKYO UNIV., SAGAMI, KANAGAWA 199-01, JAPAN.  
SOURCE: BIOCHIM BIOPHYS ACTA, (1984) 802 (1), 135-140.  
CODEN: BBACAQ. ISSN: 0006-3002.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB Affinophoresis (Shimura, et Kasai, 1982) is a newly devised electrophoretic separation technique for biomolecules, using an affinophore. The affinophore is a macromolecular polyelectrolyte bearing affinity ligands. It migrates rapidly in an electric field, and molecules which have affinity for the ligand are carried with it and separated from other molecules. An anionic affinophore for trypsin was synthesized. p-Aminobenzamidine, a competitive inhibitor of trypsin, was coupled to 1/5 of the carboxyl groups of polyacrylyl-.beta.-alanyl-.beta.-alanine by the use of water-soluble carbodiimide and the residual carboxyls were converted to sulfonate groups by coupling with aminomethanesulfonic acid. Affinophoresis was carried out in 1% agarose gel plates, and the protein bands were detected with Coomassie brilliant blue R250. Enhanced migrations of bovine and **Streptomyces griseus trypsins** towards the anode were observed with the anionic affinophore. The migrations of inactive forms prepared by active site modifications were scarcely affected. The affinophore was not effective for *S. erythraeus* trypsin, an anionic trypsin, probably because of ionic repulsion between the anionic molecules. **S. griseus trypsin** was separated from **pronase** and affinophoresis.

L12 ANSWER 4 OF 8 MEDLINE DUPLICATE 2  
ACCESSION NUMBER: 83108796 MEDLINE  
DOCUMENT NUMBER: 83108796 PubMed ID: 6185472  
TITLE: Affinophoresis of trypsins.  
AUTHOR: Shimura K; Kasai K  
SOURCE: JOURNAL OF BIOCHEMISTRY, (1982 Nov) 92 (5) 1615-22.  
Journal code: 0376600. ISSN: 0021-924X.  
PUB. COUNTRY: Japan  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198303  
ENTRY DATE: Entered STN: 19900318

Last Updated on STN: 19900318

Entered Medline: 19830311

AB We devised a new separation technique for the protein, "affinophoresis," which is based on its specific affinity and utilizes electrophoresis. This technique requires a carrier macromolecule, "affinophore," which contains both an affinity ligand for a certain protein and many charges, either positive or negative, in order to migrate rapidly in an electric field. When a mixture of proteins is electrophoresed in the presence of the affinophore, the protein having an affinity with the ligand will form a complex with the affinophore. This results in a change in the apparent electrophoretic mobility. If the protein is sufficiently accelerated, we can separate it from other materials. A cationic affinophore for trypsin was prepared. Soluble dextran MW approximately 10,000) was coupled with a DEAE-group and m-aminobenzamidine, a competitive inhibitor of trypsin. Electrophoresis of trypsins from several origins on agarose gel plates in the presence of the affinophore showed that affinophoresis actually occurred. The electrophoretic mobilities of trypsins increased towards the cathode, the same direction as the affinophore movement. The presence of leupeptin and treatment of the trypsins with TLCK suppressed the effect of the affinophore. **Streptomyces griseus trypsin**, contained in **Pronase**, was easily separated and detected. This procedure is distinct from affinity chromatography and so-called affinity electrophoresis in that the support of the affinity ligand moves, and has advantages especially for analytical purposes: for example, the detection of specific molecules regardless of their isoelectric points.

L12 ANSWER 5 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 1978:167970 BIOSIS  
DOCUMENT NUMBER: BA65:54970  
TITLE: THE EFFECTS OF PROTEASES ON PROTEINS AND GLYCO PROTEINS OF DICTYOSTELIUM-DISCOIDEUM PLASMA MEMBRANES.  
AUTHOR(S): PARISH R W; SCHMIDLIN S; MUELLER U  
CORPORATE SOURCE: CYTOL., INST. PLANT BIOL., UNIV. ZUER., 8008 ZUERICH, SWITZ.  
SOURCE: EXP CELL RES, (1977 (RECD 1978)) 110 (2), 267-276.  
CODEN: ECREAL. ISSN: 0014-4827.  
FILE SEGMENT: BA; OLD  
LANGUAGE: English

AB D. discoideum cells were incubated with proteases, the plasma membranes subsequently **isolated** and changes in proteins and glycoproteins **examined** with dodecylsulfate gel electrophoresis. Low papain concentrations gave rise to a protein band which apparently derived from actin. Since actin was the only protein attacked, the results suggest some part of the actin is exposed on the outer surface of the cell. Higher papain concentrations released a substantial portion of actin from the plasma membrane and partially digested some of the glycoproteins. Since the new actin-derived band was not further digested, the glycoproteins may be required to stabilize the actin polymer rather than anchor those actin molecules which are directly associated with the plasma membrane. **Pronase** [*Streptomyces griseus*] treatment released the 2 myosin heavy chains from the plasma membrane, in particular the higher MW chain. Actin was not affected. Some glycoproteins were digested. Trypsin attacked many of the plasma membrane proteins, and the myosin heavy chains were completely removed. Actin was only moderately affected. However, the glycoproteins were entirely resistant to trypsin. Apparently the myosin heavy chains are attacked either due to their partial exposure on the cell surface or the exposure of proteins which anchor them in the membrane. These anchoring proteins cannot be glycoproteins or actin. Proteins and glycoproteins were largely digested when **isolated** plasma

membranes were incubated with papain and **pronase**. The effects of trypsin on whole cells and **isolated** plasma membranes were similar.

L12 ANSWER 6 OF 8 MEDLINE DUPLICATE 3  
ACCESSION NUMBER: 76213113 MEDLINE  
DOCUMENT NUMBER: 76213113 PubMed ID: 819428  
TITLE: Affinity chromatography of trypsin and related enzymes.  
III. Purification of **Streptomyces griseus trypsin** using an affinity adsorbent containing a tryptic digest of **protamine** as a ligand.  
AUTHOR: Yokosawa H; Hanba T; Ishii S  
SOURCE: JOURNAL OF BIOCHEMISTRY, (1976 Apr) 79 (4) 757-63.  
Journal code: 0376600. ISSN: 0021-924X.  
PUB. COUNTRY: Japan  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 197609  
ENTRY DATE: Entered STN: 19900313  
Last Updated on STN: 19900313  
Entered Medline: 19760902

AB A new, simple method has been developed for the **purification** of **Streptomyces griseus trypsin** [EC 3.4.21.4] from **Pronase**. Only a single operation of affinity chromatography on an agarose derivative, which was easily prepared by coupling a tryptic digest of salmine to cyanogen bromide-activated Sepharose 4B, was required. A high degree of homogeneity was demonstrated for the **purified** enzyme by disc electrophoresis, SDS-polyacrylamide gel electrophoresis and gel filtration, as well as by active-site titration. The behavior of a carboxypeptides B [EC 3.4.12.3]-like enzyme present in **Pronase** is also discussed.

L12 ANSWER 7 OF 8 MEDLINE  
ACCESSION NUMBER: 75127939 MEDLINE  
DOCUMENT NUMBER: 75127939 PubMed ID: 235280  
TITLE: Enzymic and physicochemical properties of **Streptomyces griseus trypsin**.  
AUTHOR: Olafson R W; Smillie L B  
SOURCE: BIOCHEMISTRY, (1975 Mar 25) 14 (6) 1161-7.  
Journal code: 0370623. ISSN: 0006-2960.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 197507  
ENTRY DATE: Entered STN: 19900310  
Last Updated on STN: 19950206  
Entered Medline: 19750707

AB **Streptomyces griseus trypsin** has been **isolated** from **Pronase** by ion-exchange chromatography on CM-Sephadex and SE-Sephadex. The **isolated** enzyme was homogeneous by the criteria tested except for a low degree of contamination by an enzyme with nontryptic activity. The latter could be partially resolved by chromatography on Bio-Rex 70. The molar absorbandy at 280 nm was found to be 3.96 times 10<sup>-4</sup> M<sup>-1</sup>/cm and the Elcm1% was found to be 17.3. The molecular weight was 22,800 plus or minus 800. The enzyme was found to be stable at 0 degrees from pH 2 to 10. At 30 degrees the enzyme was maximally stable at pH 3-4 and significantly stabilized in the neutral and

alkaline range by 15 mM Ca<sup>2+</sup>. Some evidence was obtained for a reversible denaturation of the enzyme at pH 12.0 and 2.0. The K<sub>m</sub> for N-alpha-benzoyl-L-arginine ethyl ester at pH 8.0 in 20 mM CaCl<sub>2</sub>-0.1 M KCl-10 mM Tris-HCl buffer at 30 degrees was found to be 7.7 plus or minus 1.9 times 10<sup>-6</sup> M and the esterase activity was observed to be dependent on an ionizing group with pK<sub>a</sub> equals 5.85. In 2H<sub>2</sub>O this pK<sub>a</sub> was increased to 6.35 and the rate of hydrolysis decreased threefold. The rate of hydrolysis was independent of pH between 8 and 10. The inhibition of the enzyme with L-1-chloro-3-tosylamido-4-phenyl-2-butanone was shown to be associated with the alkylation of its single histidine residue. This residue is present in a homologous amino acid sequence as the active-site histidine in trypsin and chymotrypsin. Optical rotatory dispersion and circular dichroism measurements over the pH range 5.3-10.5 indicated no significant conformational change until the pH was increased above 10.1. The observation that, under the conditions tested, acetylation and carbamylation of the NH<sub>2</sub>-terminal valine were incomplete is consistent with the view that this group is buried as an ion pair and only becomes available for deprotonation and reaction upon denaturation of the enzyme at pH values greater than 10.0.

L12 ANSWER 8 OF 8 MEDLINE  
ACCESSION NUMBER: 73236980 MEDLINE  
DOCUMENT NUMBER: 73236980 PubMed ID: 4199084  
TITLE: The amino acid sequence of *Streptomyces griseus* trypsin. I. The peptic peptides.  
AUTHOR: Jurasek L; Smillie L B  
SOURCE: CANADIAN JOURNAL OF BIOCHEMISTRY, (1973 Jul) 51 (7) 1077-88.  
PUB. COUNTRY: Journal code: 0421034. ISSN: 0008-4018.  
DOCUMENT TYPE: Canada  
LANGUAGE: Journal; Article; (JOURNAL ARTICLE)  
FILE SEGMENT: English  
ENTRY MONTH: Priority Journals  
ENTRY DATE: 197310  
Entered STN: 19900310  
Last Updated on STN: 19900310  
Entered Medline: 19731015